



Hungry and thirsty: Effects of CO₂ and limited water availability on plant performance[☆]

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ABSTRACT

Carbon dioxide and water are crucial resources for plant growth. With anthropogenic fossil fuel emissions, CO₂ availability is and has been increasing since the last glacial maximum. Simultaneously water availability is expected to decrease and the frequency and severity of drought episodes to increase in large parts of the world. How plants respond to these two changes will help in understanding plants' responses to climate of the future. Here we sought to understand how drought affects plant traits responses to CO₂ and whether there are trade-offs in responsiveness to low and elevated CO₂ and drought. We grew seedlings of seven C₃ annuals at past low (160 μl l⁻¹), ambient (450 μl l⁻¹) and elevated (750 μl l⁻¹) CO₂. At each concentration plants were subjected to well-watered conditions (100% soil water availability, SWA), 40% SWA or 20% SWA. We measured biomass allocation, relative growth rate, tissue N concentration, and gas exchange. Compared to well-watered conditions plant size was an important element in the absolute response to SWA decrease, i.e. the smaller, slow growing species were unaffected by drought at low CO₂. Plants allocated less mass to root tissue at low CO₂ contrasting with increased root mass fraction at lower SWA at ambient CO₂. Across all traits measured, we found mostly additive effects of CO₂ and water. As due to climate change regions become more drought prone these results suggest CO₂ fertilization will not counteract the effects of reduced water availability.

1. Introduction

Plant growth is limited by the availability of light, nutrients, water and carbon. In terms of carbon acquisition, the growth rate of plants can be separated into a morphological and a physiological component (Evans, 1972). Several meta-analyses have shown that both morphological traits, such as specific leaf area (SLA; leaf area per dry mass), leaf mass fraction (LMF; leaf mass per plant mass) and root mass fraction (RMF; root mass per plant mass) and physiological traits, such as photosynthetic rate, nitrogen concentration and carbon concentration, have a strong plastic response to elevated CO₂ (Poorter and Navas, 2003; Ainsworth and Long, 2005; Norby and Zak, 2011). Interestingly, there is an even stronger response of these traits to low CO₂, representative of atmospheric composition in the distant past (Temme et al., 2013). However, environmental changes both in the past and in

the future were, are and will be multivariate. Thus, how the availability of CO₂ and that of other resources interact is a key question for understanding the full response of plants to CO₂.

Considering the full trajectory from past low, to current, to future high CO₂ concentrations aids in understanding plant response to CO₂. Anthropogenic emissions will likely increase current carbon dioxide concentrations from 410 μl l⁻¹ to an estimated 600–900 μl l⁻¹ by the end of the century (Meinshausen et al., 2011). In plants' recent geologic past CO₂ levels have risen from a >2 Myr period of low CO₂, ≈180 μl l⁻¹ during the Pleistocene glacials (Hönisch et al., 2009), to today's 410 μl l⁻¹ (Keeling et al., 2005) after the Industrial Revolution. In the light of plants' evolutionary history in low CO₂ and the current fast shift to high CO₂, understanding plants' responses to water and carbon availability over the full range of CO₂ could aid in predicting their responses to the future climate and atmospheric composition (Tissue and Lewis, 2012).

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Carbon and water fluxes are tightly linked through the stomata. This underpins the potential for interaction between CO₂ and water availability. At elevated CO₂, transpiration is reduced transiently due to closing stomata (Ainsworth and Rogers, 2007) or developmentally by a lower number, or density, of stomata (Haworth et al., 2013). Elevated CO₂ also reduces the nitrogen concentration in plants (Ainsworth and Long, 2005). One of several mechanisms hypothesized to cause this is a reduced flux of water across the roots due to reduced transpiration (Feng et al., 2015). Thus plant responses to limit water loss, by closing stomata, can affect performance at elevated CO₂ indicating the potential for trade-offs between plant responses to carbon and water availability.

Given the rapid increase in CO₂ concentration in the atmosphere and a changing climate, a lot of research has been done on how plants respond to elevated CO₂ and how water availability interacts with that response. For example, in forest plots canopy water use has been found to be elevated in response to elevated CO₂ in developing stands, but reduced in established stands (Warren et al., 2011). In climate chambers, the CO₂ fertilization effect has been found to be relatively large under dry conditions (Poorter and Pérez-Soba, 2001). In contrast, in the field drought tolerance is not necessarily increased. In a desert environment, elevated CO₂ was hypothesized to increase productivity due to increased water use efficiency. However, after 10 years of elevated CO₂ productivity and community composition remained unaltered (Newingham et al., 2013; Smith et al., 2014). Here the strong water limitation led to no stimulation by elevated CO₂.

While the majority of research has been done on predicting plants' response to future environmental conditions, understanding plants' response to conditions of the past might provide clues about possible physiological or morphological constraints in response to future conditions. Low CO₂ has profound impacts on plant traits and plant performance, including a strong reduction in biomass and growth rate, higher specific leaf area (SLA, i.e. thinner or less dense leaves), larger leaf mass fraction (leaf mass per plant mass) and strongly increased nitrogen concentration (Gerhart and Ward, 2010; Temme et al., 2013, 2015, 2017; Becklin et al., 2014). Leaf traits are adjusted in such a way as to move towards the resource acquisitive end of the across-species leaf economic spectrum (Wright et al., 2004; Reich, 2014) as expressed by higher leaf mass fraction, higher SLA and higher nitrogen content (Temme et al., 2017). However, this suite of traits is associated with lesser drought tolerance (Hallik et al., 2009; Ouédraogo et al., 2013). This strongly suggests that there could be a trade-off between adaptations to low versus high CO₂ and those to drought.

Owing to the technical hurdles associated with growing plants at low CO₂, only limited studies have assessed drought effects on plant functioning at past low CO₂ (Gerhart and Ward, 2010). Plant performance at dry conditions in low CO₂ shows contrasting responses depending on the species and the experiment. For example, *Sequoia* trees had greater xylem hydraulic failure, increased mortality and reduced defensive compounds at low CO₂ (Quirk et al., 2013). Yet, in *Phaseolus vulgaris* drought tolerance was increased at low CO₂ due to improved xylem functioning (Medeiros and Ward, 2013). Moreover, the smaller size of plants at low CO₂ might prove beneficial during periods of reduced precipitation as they take up less water and may deplete a given water supply more slowly within a limited soil volume (Liu et al., 2016).

In general plants balance resource uptake such that growth is equally limited by all resources (Bloom and Mooney, 1985; Chapin et al., 1987). Here we aim to reveal overall patterns of plant traits and growth performance response to CO₂ and water availability regimes, addressing: (1) how CO₂ concentration from past to future and soil water availability interact to affect plant performance and (2) whether there are trade-offs in the growth responsiveness to CO₂ – including plant size – and to drought.

We sought to answer these questions by experimentally growing seedlings of seven annual C₃ herbaceous species broadly ranging in responsiveness to CO₂ and differing in specific leaf area and leaf mass fraction at past low, ambient and future high CO₂ and at a broad range

of soil water availability (SWA).

2. Material and methods

We grew seedlings of 7 different C₃ herbaceous species, including grasses (G), forbs (F) and N₂-fixers (NF), at three levels of carbon and water supply in a fully factorial design. Species grown were *Agrostis capillaris* L. (G), *Clinopodium chinense* (Benth.) Kuntze (F), *Hemisteptia lyrata* (Bunge) Fisch. & C.A.Mey. (F), *Medicago lupulina* L. (NF), *Rumex chalepensis* Mill. (F), *Stellaria media* (L.) Vill. (F) and *Vicia sepium* L. (NF), i.e. a subset of the species in Temme et al. (2015, 2017). These species, from provenances in temperate Europe and subtropical China, were selected based on the broad range in allocation, N uptake strategy and leaf traits they represented.

Plants were grown in three controlled-environment walk-in chambers (Reftech bv, Sassenheim, NL) at the Phytotron labs at Utrecht University, The Netherlands, at which we kept CO₂ at low, ambient and high level respectively following Temme et al. (2015, 2017). CO₂ in the low chamber was kept at a low 160 µl l⁻¹ by scrubbing ambient air of CO₂ down to target level with a molecular sieve (PG 1500 L, CMC Instruments GmbH, Eschborn). The ambient CO₂ chamber was not directly controlled for CO₂. Concentration there was found to be 450 µl l⁻¹, likely due to the chambers being situated inside an office building and near a major road. The elevated CO₂ chamber was kept at 750 µl l⁻¹ by adding fossil fuel derived CO₂ from pressurized canisters to ambient air ventilating the chamber. CO₂ levels were continuously monitored (GMP343, Vaisala GmbH, Bonn) with scrubber capacity and CO₂ supply adjusted accordingly. While handling plants in the low chamber, exhaled breath was captured using a gas mask connected with an airtight bag in order to limit CO₂ levels rising.

Individuals were germinated from field-collected seeds on wet filter paper or sand and two to three days after germination individuals were transferred to experimental conditions. Individuals were transplanted to 400 ml pots, one individual per pot, containing coarse river sand (containing trace amounts of clay and silt) to facilitate root washing. We did not expect pot size (i.e. volume available for rooting) to play a substantial role in plant response to CO₂ and drought. Plants were expected to remain below 1 g dry weight L⁻¹ soil volume during the experiment (Poorter et al., 2012) and the soil water content at which drought effects become apparent is not related to pot size (Ray and Sinclair, 1998). We grew 6–8 individuals per species / treatment combination.

Experimental conditions were similar to those in Liu et al. (2016). Light was set to $\approx 350 \mu\text{mol m}^{-2} \text{s}^{-1}$ with a 10-h photoperiod, at 21 °C during day, 18 °C at night and 70% relative air humidity. Up to the development of the first leaf, pots were watered three times per day from below by water automatically flowing over the bench. After that individuals were separated into three equal size groups, one of which was kept at full water supply (100% soil water availability, SWA) and two of which were subjected to drought (40% SWA and 20% SWA). Three times per week (Mo–Wed–Fri) all drought treatment pots were weighed to the nearest 0.1 g and water was added back to target SWA level. To prevent nutrient limitation 50 ml of full Hoagland solution (6 mM KNO₃, 4 mM Ca(NO₃)₂, 2 mM NH₄H₂PO₄, 1 µM KCl, 25 µM H₃BO₃, 2 µM MnSO₄, 2 µM ZnSO₄, 0.1 µM CuSO₄, 0.1 µM (NH₄)₆Mo₇O₂₄, 20 µM Fe(Na)EDTA) were added to each pot three times per week. Damage to freshly germinated individuals was prevented by slowly increasing the concentration from 25% to full Hoagland as plants grew until full development of the first leaf.

After the opening of the first leaf (t₀), a baseline harvest (wt₀) was performed. Plants were then grown for three more weeks (t₁) after which a final harvest (wt₁) was done. Relative growth rate (RGR) during the experiment was calculated following Hoffmann and Poorter (2002) as $\text{RGR} = [\ln(\text{wt}_1) - \ln(\text{wt}_0)] / (t_1 - t_0)$. At final harvest we counted dead individuals and measured live plants for root and shoot fresh weight and leaf area and fresh weight of a single representative leaf. The representative full-grown leaf was scanned (Canon LiDe 110) and

measured using ImageJ v1.47 for SLA ($m_{\text{leaf}}^2 g_{\text{leaf}}^{-1}$ dry weight). Plant material was oven dried for >48 h at 70 °C after which root, shoot and leaf dry mass were determined. Leaves were subsequently removed from stems, or other stem-like tough tissue including petioles, and stem dry mass determined. From these mass data we calculated leaf mass fraction (LMF, $g_{\text{leaf}} g_{\text{plant}}^{-1}$), root mass fraction (RMF, $g_{\text{root}} g_{\text{plant}}^{-1}$) and stem mass fraction (SMF, $g_{\text{stem}} g_{\text{plant}}^{-1}$). Leaf material was ground up in a ball mill (MM200, Retsch, Haan, DE) and leaf C and N concentration determined by flash combustion using a Perkin Elmer 2400 CHNS analyzer (Thermo Scientific, Rodana, IT).

For a subset of species at ambient and elevated CO₂ leaves were large enough to allow that a day prior to harvest we could measure gas exchange using a LI-6400 (LICOR, Nevada, USA). Gas exchange was carried out following Temme et al. (2017). Briefly, one fully developed leaf per individual was placed to acclimate in the cuvette, set similar to growth conditions, for two minutes in the light (red-blue light source, LI-6400-02B). The area inside the leaf cuvette was marked and removed at final harvest. When leaves did not fill the total cuvette area (6 cm²) the portion of the leaf that could be placed inside the cuvette was scanned using a Canon LiDe 110 scanner. Leaf area was then measured using ImageJ v1.47. Net area-based photosynthesis (A_{net}) and respiration rates (R) and stomatal conductance (g_s) were then calculated by using the correct area in the gas analyser equations.

Data analysis and statistics were carried out using R version 3.12 (R Core Team, Vienna, Austria) as in Temme et al. (2015). To limit the effect of pseudoreplication (only one climate chamber per CO₂ level), we took the individual species mean responses as replicates in all analyses on trait responses to CO₂ and soil water content (SWA). Trait responses to CO₂ and SWA were viewed both in absolute terms and relative to ambient CO₂. For relative responses trait values were log_e transformed prior to analysis. This approach has the benefit that a halving or a doubling in trait value from ambient CO₂ has the same transformed difference. Trait responses to CO₂ and SWA were tested via two-way ANOVA, as shown in Appendix table 1. Within each CO₂ or SWA level differences between groups were tested by a Tukey test corrected for multiple comparisons. Association between traits and trait responses were tested via Standardized major axis (SMA) regression (Warton et al., 2012).

3. Results

Plants were strongly impacted by drought at all CO₂ levels. At the lowest level of soil water availability (20% SWA) some individuals of most species died, except in *Agrostis capillaris* and *Vicia sepium*. For *Clinopodium chinense* and *Medicago lupulina* this only happened at low CO₂, for *Rumex* only at high and low CO₂, for *Stellaria* only at ambient CO₂ and for *Hemisteptia* at all CO₂ levels.

3.1. Drought and CO₂ effects on plant performance and traits

Increased carbon availability from 160 $\mu\text{l l}^{-1}$ to 450 $\mu\text{l l}^{-1}$ and 750 $\mu\text{l l}^{-1}$ CO₂ resulted in larger overall plant biomass at well-watered and moderately dry water levels. CO₂ concentration and SWA interacted in such a manner that at very low CO₂ concentration (160 $\mu\text{l l}^{-1}$) increased drought had no significant effect on biomass (Fig. 1a). Relative to ambient CO₂ the effect of decreased CO₂ and increased CO₂ was the same at all SWA levels (Fig. 1b). Response to CO₂ was varied across species resulting in low power to detect differences. With a less stringent multiple comparison penalty the relative effect of CO₂ (compared to ambient) was significantly reduced biomass at low CO₂ and significantly increased biomass at elevated CO₂ and well-watered conditions. Relative growth rate (RGR, $g g^{-1} d^{-1}$) was affected by CO₂ comparable to plant biomass. Between low CO₂ and high CO₂ RGR was significantly increased at well-watered and moderately dry levels (Fig. 2a). SWA and CO₂ did not show a significant interaction for RGR.

Biomass allocation to roots and leaves was affected by both CO₂ and

SWA. Lower SWA led to increased root mass fraction (RMF) at ambient CO₂ but not at low or high CO₂. Additionally, CO₂ increase from low to ambient concentration substantially increased RMF (Appendix Fig. 1). Leaf mass fraction was significantly increased at low CO₂ but only under severe water limitation (Appendix Fig. 2).

The scaling relationship between log-transformed leaf biomass and root biomass did not differ between water treatments, but there was a difference in elevation between CO₂ levels (Fig. 3). Standardized major axis (SMA) regression showed a similar slope between CO₂ levels when all water treatments were pooled. The scaling relationship between leaf and root biomass at ambient and high CO₂ was the same, with plants at high CO₂ being moved along the common axes indicating the stimulating effect of CO₂ on plant biomass. However, the scaling slope at low CO₂ had a higher intercept than at ambient and high CO₂ ($p < 0.01$) (Fig. 3). Thus for a given leaf biomass plants grown at low CO₂ had a lower root biomass.

Leaf traits were affected by CO₂ and SWA as well. Specific leaf area (SLA) was strongly affected by CO₂ with species increasing their SLA from ambient and high to low CO₂ (Appendix Fig. 3). SWA only affected SLA at low CO₂ where drought led to plants having a lower SLA. Nitrogen concentration per unit mass (N%) was strongly affected by low CO₂ with higher N levels at lower CO₂ ($p < 0.001$), while it was not affected by SWA (Appendix Fig. 4).

Leaf gas exchange data under reduced SWA could only be obtained from plants grown at ambient and high CO₂. Leaves of individuals at low CO₂ and 20%–40% SWA were too small to fit the LiCOR 6400 cuvette, so the interaction of CO₂ and SWA could not be tested at low CO₂. In absolute terms photosynthesis (A_{net}) was stimulated by increasing CO₂ from low to ambient. At ambient and high CO₂ reducing SWA led to lower A_{net} ($p < 0.05$) (4a). Stomatal conductance (g_s) was not affected by CO₂ though plants did show a strong decrease in g_s at lower SWA (Fig. 4b). A_{net} and g_s combined in intrinsic water use efficiency (iWUE, A_{net}/g_s) being significantly higher at reduced water availability, though more so at ambient than high CO₂ (Fig. 4c).

3.2. Trade-offs in biomass response to CO₂ and water

Trade-offs in response to CO₂ and soil water availability were not readily apparent. At low CO₂ there was no relationship between the extent at which carbon starvation decreased biomass compared to ambient CO₂ and the effect of reduced soil water availability on biomass compared to that at 100% SWA at low CO₂. Thus, plants that could cope well with drought stress were not affected differently by reduced carbon concentration (Appendix Fig. 5 a). Species that were stimulated more by elevated CO₂ also tended to be more affected by reduced SWA (Appendix Fig. 5b). There was a significant relationship between plant biomass and biomass reduction due to drought (Fig. 5, $R^2 = 0.68$, $p < 0.001$). Thus, in absolute and relative terms larger plants were more affected by drought than smaller plants. Taken together this suggests that the large plants that were stimulated most by elevated CO₂ were the most negatively affected by drought.

4. Discussion

There are many potential interactive effects between carbon gain and water loss. We investigated how CO₂ affected plant performance from well-watered to severely droughted conditions and if there were trade-offs in the growth responsiveness to CO₂ versus that to drought. In agreement with earlier work we found that plant growth and biomass were strongly reduced at low CO₂ (Gerhart and Ward, 2010) and stimulated by high CO₂ (Poorter and Navas, 2003). Drought led to a similar relative reduction of plant biomass at all CO₂ levels. However, due to the carbon fertilisation effect of increasing CO₂, plants accumulated more biomass in absolute terms at higher CO₂ levels. Thus, the absolute effect of drought was in fact greater at higher CO₂ because plants could grow larger at well-watered conditions. This shows that plant biomass appears to be a key element in the responsiveness to soil

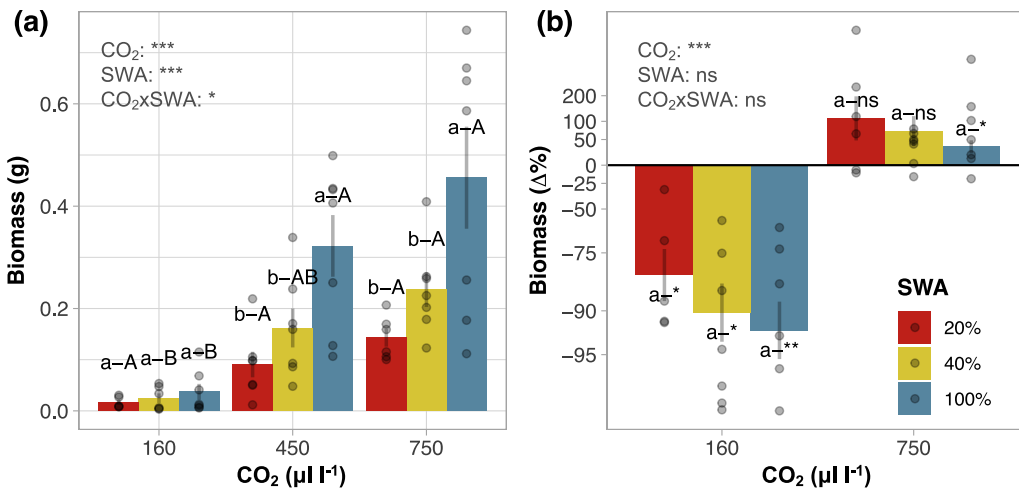


Fig. 1. Plant biomass in relation to CO_2 concentration and soil water availability (SWA) in (a) absolute terms and (b) relative to ambient CO_2 . Grey dots indicate species average biomass ($n = 4-6$ per species), grey bars denote standard error. Letters denote Tukey post-hoc tests of difference between SWA levels (lowercase) and difference between CO_2 levels (uppercase). In panel (b) instead of comparing CO_2 levels the difference from zero, no effect, is tested. Inset shows two-way ANOVA of CO_2 and SWA and their interaction (see Appendix Table 1 for full model output). ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

water availability (SWA) at past low, ambient and future high CO_2 .

4.1. Interactive effects of carbon and water

Compared to ambient CO_2 we found no increase in CO_2 stimulation of plant biomass under dry conditions, which contrasts with an earlier study (Poorter and Pérez-Soba, 2001), but is consistent with some field results from arid ecosystems (Smith et al., 2014). However we did find when taking size into consideration, that plants that grew larger at high CO_2 and ample water were more affected by reduced SWA (Fig. 5), as Liu et al. (2016) found for the grass *Avena sativa* and the forb *Chenopodium album*. Moreover, lower SWA at elevated CO_2 reduced the effect of CO_2 fertilization on biomass production. When SWA is low it seems that excess available carbon cannot be used and the stimulating effect of elevated CO_2 disappears. Furthermore, we found that the allometric relationship between leaf and root biomass was different at low CO_2 from that at ambient and high CO_2 (Fig. 3). For a given leaf mass, plants had less root mass at low CO_2 . More extreme drought than in this study could exacerbate negative effects on growth at low CO_2 as low root mass allocation is linked to poor drought tolerance (Zwicke et al., 2015).

4.2. Trade-offs in responsiveness to CO_2 and water

In this short-term study we found no clear evidence for trade-offs in the responses to water versus to CO_2 (Appendix Fig. 5). Species that could tolerate lower SWA did not respond differently to either elevated or reduced CO_2 . However with more species or longer drought duration

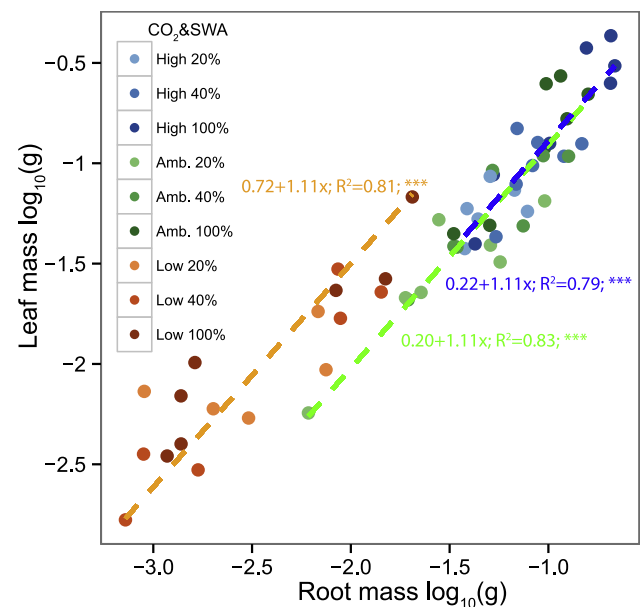


Fig. 3. Allometric relationship between leaf and root biomass at low ($160 \mu\text{l l}^{-1}$), ambient ($450 \mu\text{l l}^{-1}$), and high ($750 \mu\text{l l}^{-1}$) CO_2 . Lines indicate proportional (log10) scaling slope of leaf biomass to root biomass at each CO_2 concentration based on SMA regression. ***: $p < 0.001$.

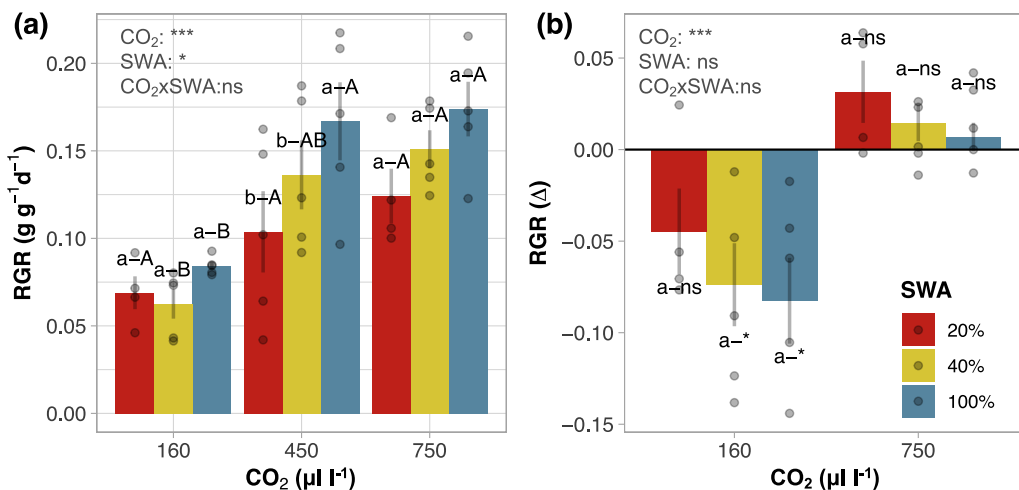


Fig. 2. Plant relative growth rate (RGR) in relation to CO_2 concentration and soil water availability (SWA) in (a) absolute terms and (b) relative to ambient CO_2 . Grey dots indicate species average RGR ($n = 4-6$ per species), grey bars denote standard error. Letters denote Tukey post-hoc tests of difference between SWA levels (lowercase) and difference between CO_2 levels (uppercase). In panel (b) instead of comparing CO_2 levels the difference from zero, no effect, is tested. Inset shows two-way ANOVA of CO_2 and SWA and their interaction (see Appendix Table 1 for full model output). ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

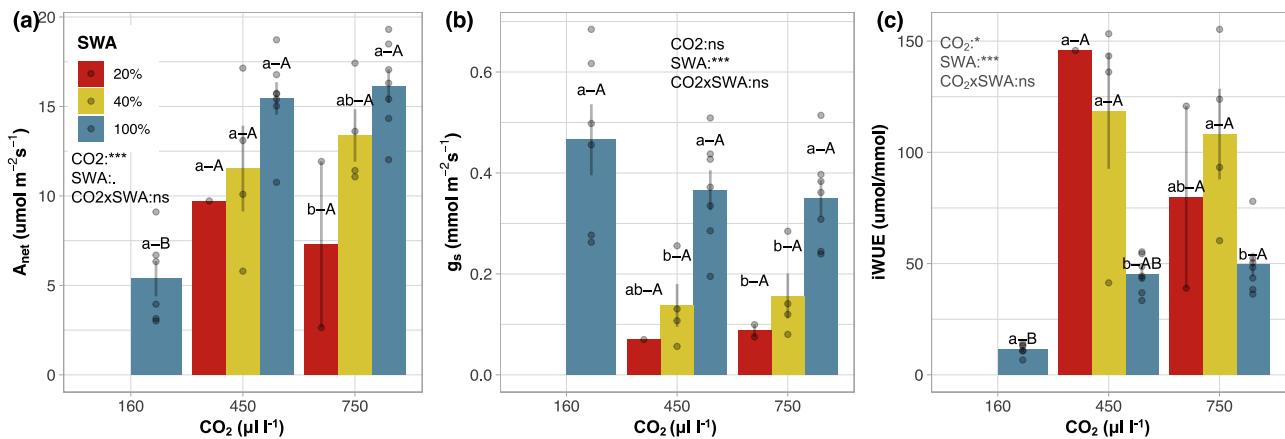


Fig. 4. Effect of soil water availability (SWA) and CO₂ concentration, on (a) net photosynthesis (A_{net}), (b) stomatal conductance (g_s) and (c) intrinsic water use efficiency (iWUE). Grey dots indicate species average value ($n = 4-6$ per species), grey bars denote standard error. Letters denote Tukey post-hoc tests of difference between SWA levels (lowercase) and difference between CO₂ levels (uppercase). Inset shows two-way ANOVA of CO₂ and SWA and their interaction (see Appendix Table 1 for full model output). ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

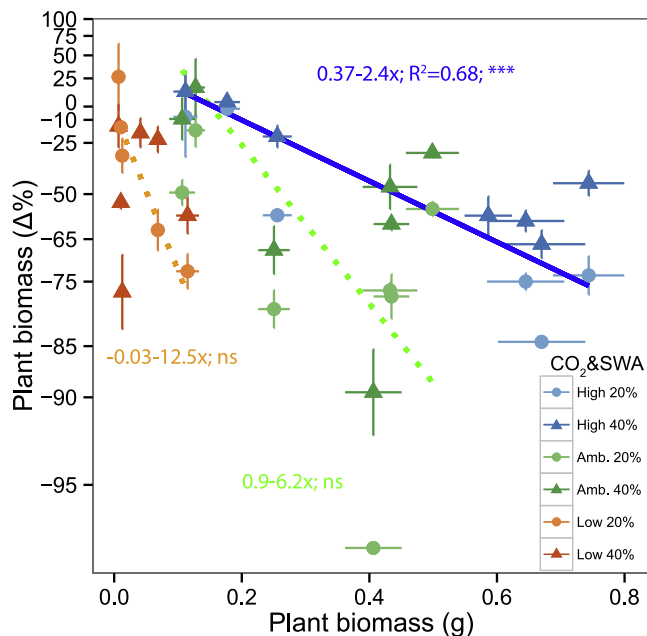


Fig. 5. Effect of plant size at well-watered conditions on the level of biomass reduction due to soil water availability (SWA) at low ($160 \mu\text{l l}^{-1}$), ambient ($450 \mu\text{l l}^{-1}$) and high ($750 \mu\text{l l}^{-1}$) CO₂. Lines indicate SMA regression between biomass and biomass effect at reduced SWA at low CO₂ (red), ambient CO₂ (green) and high CO₂ (blue). Dashed lines: non-significant slope. ***: $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

these trade-offs, if present, may become apparent. Plant species with high growth rates are stimulated more by elevated CO₂ (Cornelissen et al., 1999; Poorter and Navas, 2003) and more affected by reduced CO₂ (Temme et al., 2015). Traits associated with high drought tolerance (high RMF, low SLA) are negatively associated with plant growth rates (Reich, 2014). Potentially plants with reduced growth rates due to adaptations to poor water conditions could result in a reduced stimulation by elevated CO₂ at well-watered conditions.

From a competition point of view, the greater stimulation of fast growers with traits beneficial at low levels of drought could help them in outcompeting drought tolerant slow growers at sufficient water supply. However, in a model that incorporates nutrient use and capture it was found that elevated CO₂ led to increased coexistence between species due to reduced competitive ability and increased evenness

because resources were more evenly distributed (Ali et al., 2015). Field experiments combining CO₂ and drought would be an excellent way to see if plants exhibit the same response to water as modelled for nutrients; and how, as in our study, plant size modulation of drought effects affects this response.

4.3. Recommendations and experimental considerations

The role plant size had in modulating the effect of SWA is likely influenced by our drought scenario. Drought effects can be investigated using many different scenarios (Tardieu, 2012; O'Grady et al., 2013; He and Dijkstra, 2014). Here we achieved drought stress experimentally by subjecting plants to reduced water addition. SWA was kept at 20% and 40% of fully watered conditions by adding water to the desired level three times a week. Locally in the soil SWA was then inevitably higher than the average SWA. For small plants, these short time periods where they had access to water may have been enough to maintain functioning. As larger plants need more water they draw down soil water supply more quickly and as such are more affected by reduced precipitation (Liu et al., 2016).

Longer term drought combined with carbon starvation as in the past could have had more detrimental effects on plant growth and performance (Hartmann et al., 2013). At high CO₂ changes in plant morphology and physiology could also modulate the effects of longer term drought (Sperry and Love, 2015). CO₂ starvation leads to plants with high SLA and low root mass fraction (Temme et al., 2015) which is opposite to what would be beneficial under drought conditions (Hallik et al., 2009; Ouédraogo et al., 2013). Thus longer dry periods could show trade-offs as, in the long term, there could be opposite responses to CO₂ and water. Moreover, long term periods of elevated CO₂ and environmental stress could lead to evolutionary adaptations different from the plastic response to rapid shifts in CO₂, as was shown for natural CO₂ vents (Onoda et al., 2009).

Our results corroborate earlier findings that herbaceous C₃ species, when grown at elevated CO₂, are not more drought tolerant than at ambient CO₂ (Medeiros and Ward, 2013) and that the relative effect of drought is not greater at low or high CO₂ (Ward et al., 1999). However, the results from an earlier study with the tree *Sequoia sempervirens* (Quirk et al., 2013) are markedly different from our and previous results based on herbaceous species. This study found that low CO₂ led to increased drought stress in the form of greater mortality and slower growth. Though it should be noted that plants' different first appearance in the geological record and thus different past experience with fluctuating CO₂ levels on geological timescales could impact their response to varying CO₂ this does raise the question if availability of

water and carbon affects (slow growing) woody plants species differentially from (fast-growing) herbaceous plants.

5. Conclusion

Taken together these results paint a picture of limited interactive effects of CO₂ and water availability. Across all traits measured, we found mostly additive effects of CO₂ and water. However, it should be noted that the already stressed plants at low CO₂ did not experience extra stress due to reduced water availability. As due to climate change regions become more drought prone these results suggest CO₂ fertilization will not counteract the effects of reduced water availability.

Author contributions

AAT, JCL, WKC, RA and JHCC conceived and designed the study, AAT and JCL grew plants and collected the data, AAT, JCL, RA, JHCC analysed and interpreted the data. AAT drafted the manuscript with substantial revision by RA and JHC. All authors agree on the final text. AAT takes responsibility for the integrity of the work as a whole, from inception to finished article.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.flora.2018.11.006>.

References

- Ainsworth, E.A., Long, S.P., 2005. What 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytol.* 165, 351–372.
- Ainsworth, E.A., Rogers, A., 2007. The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant Cell Environ.* 30, 258–270.
- Ali, A.A., Medlyn, B.E., Aubier, T.G., Crous, K.Y., Reich, P.B., 2015. Elevated carbon dioxide is predicted to promote coexistence among competing species in a trait-based model. *Ecol. Evol.* 5, 1–18.
- Becklin, K.M., Medeiros, J.S., Sale, K.R., Ward, J.K., 2014. Evolutionary history underlies plant physiological responses to global change since the last glacial maximum. *Ecol. Lett.* 17, 691–699.
- Bloom, A.J., Mooney, H.A., 1985. Resource limitation in plants – an economic analogy. *Annu. Rev. Ecol. Syst.* 16, 363–392.
- Chapin, F.S., Bloom, A.J., Field, C.B., Waring, R.H., 1987. Plant responses to multiple environmental factors. *BioScience* 37, 49–57.
- Cornelissen, J., Carnelli, A., Callaghan, T., 1999. Generalities in the growth, allocation and leaf quality responses to elevated CO₂ in eight woody species. *New Phytol.* 141, 401–409.
- Evans, G.C., 1972. *The Quantitative Analysis of Plant Growth*. Blackwell Scientific, Oxford.
- Feng, Z., Rütting, T., Pleijel, H., Wallin, G., Reich, P.B., Kammann, C.I., Newton, P.C.D., Kobayashi, K., Luo, Y., Uddling, J., 2015. Constraints to nitrogen acquisition of terrestrial plants under elevated CO₂. *Glob. Chang. Biol.* 21, 3152–3168.
- Gerhart, L.M., Ward, J.K., 2010. Plant responses to low [CO₂] of the past. *New Phytol.* 188, 674–695.
- Hallik, L., Niinemets, Ü., Wright, I.J., 2009. Are species shade and drought tolerance reflected in leaf-level structural and functional differentiation in Northern Hemisphere temperate woody flora? *New Phytol.* 184, 257–274.
- Hartmann, H., Ziegler, W., Kolbe, O., Trumbore, S., 2013. Thirst beats hunger – declining hydration during drought prevents carbon starvation in Norway spruce saplings. *New Phytol.* 200, 340–349.
- Haworth, M., Elliott-Kingston, C., McElwain, J.C., 2013. Co-ordination of physiological and morphological responses of stomata to elevated [CO₂] in vascular plants. *Oecologia* 171, 71–82.
- He, M., Dijkstra, F.A., 2014. Drought effect on plant nitrogen and phosphorus: a meta-analysis. *New Phytol.* 204, 924–931.
- Hoffmann, W.A., Poorter, H., 2002. Avoiding bias in calculations of relative growth rate. *Ann. Bot.* 80, 37–42.
- Hönisch, B., Hemming, N.G., Archer, D., Siddall, M., McManus, J.F., 2009. Atmospheric carbon dioxide concentration across the mid-Pleistocene transition. *Science* 324, 1551–1554.
- Keeling, C.D., Piper, S.C., Bacastow, R.B., Wahlen, M., Whorf, T.P., Heimann, M., Meijer, H.A., 2005. Atmospheric CO₂ and ¹³CO₂ exchange with the terrestrial biosphere and oceans from 1978 to 2000: observations and carbon cycle implications. In: Ehleringer, J.R., Cerling, T.E., Dearing, M.D. (Eds.), *A History of Atmospheric CO₂ and Its Effects on Plants, Animals, and Ecosystems*. Springer Verlag, New York, pp. 83–113.
- Liu, J.-C., Temme, A.A., Cornwell, W.K., van Logtestijn, R.S.P., Aerts, R., Cornelissen, J.H.C., 2016. Does plant size affect growth responses to water availability at glacial, modern and future CO₂ concentrations? *Ecol. Res.* 31, 213–227.
- Medeiros, J.S., Ward, J.K., 2013. Increasing atmospheric [CO₂] from glacial to future concentrations affects drought tolerance via impacts on leaves, xylem and their integrated function. *New Phytologist* 199, 738–748.
- Meinshausen, M., Smith, S.J., Calvin, K., Daniel, J.S., Kainuma, M.L.T., Lamarque, J.-F., Matsumoto, K., Montzka, S.A., Raper, S.C.B., Riahi, K., Thomson, A., Velders, G.J.M., Vuuren, D.P.P., 2011. The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. *Clim. Change* 109, 213–241.
- Newingham, B.A., Vanier, C.H., Charlet, T.N., Ogle, K., Smith, S.D., Nowak, R.S., 2013. No cumulative effect of 10 years of elevated [CO₂] on perennial plant biomass components in the Mojave Desert. *Glob. Chang. Biol.* 19, 2168–2181.
- Norby, R.J., Zak, D.R., 2011. Ecological lessons from free-air CO₂ enrichment (FACE) experiments. *Annu. Rev. Ecol. Syst.* 42, 181–203.
- O'Grady, A.P., Mitchell, P.J.M., Pinkard, E.A., Tissue, D.T., 2013. Thirsty roots and hungry leaves: unravelling the roles of carbon and water dynamics in tree mortality. *New Phytol.* 200, 294–297.
- Onoda, Y., Hirose, T., Hikosaka, K., 2009. Does leaf photosynthesis adapt to CO₂-enriched environments? An experiment on plants originating from three natural CO₂ springs. *New Phytol.* 182, 698–709.
- Ouedraogo, D.Y., Mortier, F., Gourlet-Fleury, S., Freycon, V., Picard, N., 2013. Slow-growing species cope best with drought: evidence from long-term measurements in a tropical semi-deciduous moist forest of Central Africa. *J. Ecol.* 101, 1459–1470.
- Poorter, H., Navas, M.-L., 2003. Plant growth and competition at elevated CO₂: on winners, losers and functional groups. *New Phytol.* 157, 175–198.
- Poorter, H., Pérez-Soba, M., 2001. The growth response of plants to elevated CO₂ under non-optimal environmental conditions. *Oecologia* 129, 1–20.
- Poorter, H., Bühler, J., van Dusschoten, D., Climent, J., Postma, J.A., 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Funct. Plant Biol.* 39, 839.
- Quirk, J., McDowell, N.G., Leake, J.R., Hudson, P.J., Beerling, D.J., 2013. Increased susceptibility to drought-induced mortality in *Quercus sempervirens* (Cupressaceae) trees under Cenozoic atmospheric carbon dioxide starvation. *Am. J. Bot.* 100, 582–591.
- Ray, J.D., Sinclair, T.R., 1998. The effect of pot size on growth and transpiration of maize and soybean during water deficit stress. *J. Exp. Bot.* 49, 1381–1386.
- Reich, P.B., 2014. The world-wide “fast-slow” plant economics spectrum: a traits manifesto (ed H Cornelissen). *J. Ecol.* 102, 275–301.
- Smith, S.D., Charlet, T.N., Zitzer, S.F., Abella, S.R., Vanier, C.H., Huxman, T.E., 2014. Long-term response of a Mojave Desert winter annual plant community to a whole-ecosystem atmospheric CO₂ manipulation (FACE). *Glob. Chang. Biol.* 20, 879–892.
- Sperry, J.S., Love, D.M., 2015. What plant hydraulics can tell us about responses to climate-change droughts. *New Phytol.* 207, 14–27.
- Tardieu, F., 2012. Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. *J. Exp. Bot.* 63, 25–31.
- Temme, A.A., Cornwell, W.K., Cornelissen, J.H.C., Aerts, R., 2013. Meta-analysis reveals profound responses of plant traits to glacial CO₂ levels. *Ecol. Evol.* 3, 4525–4535.
- Temme, A.A., Liu, J.-C., Cornwell, W.K., Cornelissen, J.H.C., Aerts, R., 2015. Winners always win: growth of a wide range of plant species from low to future high CO₂. *Ecol. Evol.* 5, 4949–4961.
- Temme, A.A., Liu, J.-C., van Hal, J., Cornwell, W.K., Cornelissen, J.H.C., Aerts, R., 2017. Increases in CO₂ from past low to future high levels result in ‘slower’ strategies on the Leaf Economic Spectrum. *Perspect. Plant Ecol. Evol. Syst.* 29, 41–50.
- Tissue, D.T., Lewis, J.D., 2012. Learning from the past: how low [CO₂] studies inform plant and ecosystem response to future climate change. *New Phytol.* 194, 4–6.
- Ward, J.K., Tissue, D.T., Thomas, R.B., Strain, B.R., 1999. Comparative responses of model C₃ and C₄ plants to drought in low and elevated CO₂. *Glob. Chang. Biol.* 5, 857–867.
- Warton, D.I., Duursma, R.A., Falster, D.S., Taskinen, S., 2012. smatr 3- an R package for estimation and inference about allometric lines: The smatr 3 - an R package. *Methods Ecol. Evol.* 3, 257–259.
- Warren, J.M., Elisabeth, P., Wullschlegel, S.D., Thornton, P.E., Hasenauer, H., Norby, R.J., 2011. Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO₂. *Ecohydrology* 4, 196–210.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R., 2004. The worldwide leaf economics spectrum. *Nature* 428, 821–827.
- Zwicke, M., Picon-Cochard, C., Morvan-Bertrand, A., Prud'homme, M.-P., Voltaire, F., 2015. What functional strategies drive drought survival and recovery of perennial species from upland grassland? *Ann. Bot.* 116, 1001–1015.